Gαi2-Deficient Mice with Colitis Exhibit a Local Increase in Memory CD4$^+$ T Cells and Proinflammatory Th1-Type Cytokines[1]

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Mice with targeted deletion of the G protein Gαi2 develop an inflammatory bowel disease closely resembling ulcerative colitis. To better define disease pathogenesis, the mucosal immune system in Gαi2-deficient mice was studied. Phenotypic analysis of large intestine lamina propria lymphocytes revealed a large increase in memory CD4$^+$ T cells (CD44^{high}, CD45RB^{low}, CD62L^{low}). Furthermore, expression of the mucosal homing receptor integrin β7 was increased on mucosal, but not systemic, CD4$^+$ T cells. Analysis of cytokine production revealed a marked increase in proinflammatory Th1-type cytokines in inflamed colons, as compared with wild-type mice or Gαi2-deficient mice without colitis. Thus, IFN-γ and IL-1β levels were increased 13-fold and 30-fold, respectively, with more modest increases in IL-6 levels (5-fold) and TNF levels (2-fold). Inflamed colons of Gαi2-deficient mice also demonstrated increased IL-12 p40 mRNA levels. No increase in IL-2, IL-4, IL-5, and IL-10 was seen. Large intestinal epithelial cells in Gαi2-deficient mice with colitis were found by immunohistochemistry to express increased levels of both MHC class I and class II Ags. Colitis was associated with increased IgG levels (60-fold increase), predominantly IgG2a (135-fold increase), in large but not small intestinal secretions. This was shown by ELISPOT analysis to result from local production within the lamina propria. *The Journal of Immunology,* **1997, 158: 1068–1077.**

Despite continuous exposure to food and microbial Ags, adverse inflammatory reactions in the gastrointestinal mucosa are rare. Nevertheless, host defense against a large number of infectious diseases requires that the local mucosal immune system be able to recognize and respond to toxins, bacteria, parasites, or viruses. In inflammatory bowel disease (IBD),[6] i.e., ulcerative colitis and Crohn's disease, this normally protective immune response appears to be improperly regulated, and the highly activated effector cells cause extensive damage to the small and/or large intestine (1). While the mechanisms by which local intestinal immune responses are regulated remain poorly understood, previous studies have shown that T lymphocytes exert key regulatory functions in the intestinal mucosa. Consequently, inflammatory bowel disease may result, at least in part, from alterations in the function of these cells. Thus, experimental activation of the T cells in the human intestine lamina propria induces villus atrophy and increased proliferation of crypt cells (2, 3). Also, recent studies of mice with targeted disruption of genes coding for αβTCR, IL-2, IL-10, or MHC class II have shown that they develop intestinal inflammation resembling inflammatory bowel disease (4–6). Additional recent animal models for inflammatory bowel disease, e.g., the CD45RB T cell-reconstituted Tgε26 mice (7) and the CD4$^+$CD45R^{high} T cell SCID mouse model (8), likewise demonstrate the delicate immunologic balance within the intestinal mucosa and how many apparently different aberrations of the immune system can lead to chronic inflammation within the mucosa. Collectively these findings suggest that intact mucosal immune functions are a prerequisite for normal homeostasis in the intestinal mucosa.

G proteins are a family of GTP-binding proteins that are involved in a variety of transmembrane signaling systems as transducers (9). Recently, mice with a targeted mutation in the gene for the G protein subunit Gαi2 (GNAI2), located on chromosome 9, were generated by homologous recombination in embryonic stem cells (10). These mice develop an IBD that is clinically and pathologically similar to ulcerative colitis in humans. Similar to ulcerative colitis, histopathologically the Gαi2-deficient mice show acute and chronic mucosal inflammation with ulcerations limited to the colon, distal greater than proximal, crypt abscesses, and loss of goblet cells (10). Approximately one-third of the mice also develop invasive, nonpolypoid adenocarcinoma as early as 12 wk of age.

Several alterations in the systemic immune system were seen in these mice (10). Thymi from Gαi2-deficient mice exhibit an increased frequency of mature CD4$^+$8$^-$ or CD4$^-$8$^+$ single-positive

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Received for publication April 29, 1996. Accepted for publication October 30, 1996.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This study was supported by grants from the Crohn's and Colitis Foundation of America (G.R.H.), The Wenner-Gren Center Foundation (C.E.H.), The Swedish Medical Research Council (C.E.H.), The Tore Nilsson Foundation for Medical Research (C.E.H.), and National Institutes of Health (NIH) (L.B.).

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[6] Abbreviations used in this paper: IBD, inflammatory bowel disease; HPF, high power field; MLN, mesenteric lymph nodes; GM-CSF, granulocyte macrophage colony-stimulating factor; PGE_2, prostaglandin E_2; LTB_4, leukotriene B_4; PAF, platelet-activating factor; PE, phycoerythrin; IEL, intraepithelial lymphocytes; SFC, spot-forming cells; Gi proteins, inhibitory G proteins.

0022-1767/97/$02.00

thymocytes with enhanced proliferation in response to T cell stimuli. Both thymocytes and peripheral T cells from these mice produce substantially increased amounts of IL-2, IFN-γ, and TNF. Analysis of B cell development in the bone marrow and spleen of Gαi2-deficient mice did not reveal substantial alterations; however, plasma IgG and IgA levels were approximately twice as high in the Gαi2-deficient mice in comparison with wild-type mice. Finally, a pronounced neutrophilia was seen in peripheral blood and the spleen (10).

Of major importance for understanding the etiology and pathogenesis of colitis in these mice, and eventually in humans, is the characterization of phenotypic and functional changes that occur in the intestinal mucosa, in association with colitis. To this end, the present studies address a variety of phenotypic and functional changes occurring in the gastrointestinal mucosa of Gαi2-deficient mice.

Materials and Methods

Mice

Gαi2-deficient mice were generated by gene targeting, as described previously (10). The resulting male germ-line chimeras were bred with both C57BL/6 and 129/Sv females to maintain the mutation in a crossbred (129/Sv × C57BL/6) and an inbred (129/Sv) background, respectively. Gαi2-deficient mice bred on a 129/Sv background consistently showed a 100% incidence of colitis, manifesting severe disease by 16–20 wk. In contrast, mice bred on a 129/Sv × C57BL/6 crossbred background showed greatly diminished disease expression. This has made it possible for us to compare the immune responses in Gαi2-deficient mice with or without disease. The mice were kept in microisolator cages in the Center for Comparative Medicine facilities at Baylor College of Medicine. They were used from 6 wk up to >1 yr of age and were compared with age- and sex-matched Gαi2 +/+ or Gαi2 +/− control mice on similar background.

Intestinal secretions

Intestinal lavage samples for Ig determination were collected by a method adapted from Elson et al. (11). Small intestines (from pylorus to cecum) and large intestines (from cecum to anus) were isolated and carefully filled for 10 min with a protease inhibitor solution consisting of 0.1 mg/ml soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO), 50 mM EDTA and 1 mM PMSF (Boehringer Mannheim, Mannheim, Germany) in PBS. The intestinal content was then carefully transferred to a test tube, vigorously vortexed, and centrifuged for 10 min at 500 × *g* at 4°C. The supernatant was transferred to a microfuge tube and PMSF was added to a final concentration of 1 mM. The solution was mixed by vortexing and centrifuged at 13,000 × *g* for 15 min at 4°C. The resulting supernatant was mixed with PMSF and sodium azide to final concentrations of 1 mM and 0.01%, respectively, and incubated for 15 min at 4°C. Thereafter, 50 μl of FCS were added per ml of secretion, and the solution was centrifuged for an additional 15 min at 13,000 × *g* at 4°C. Secretions were stored at −70°C until analysis.

Lymphocyte isolation

Spleen and mesenteric lymph node lymphocytes were isolated as previously described (12) following passage of the tissue through a nylon mesh screen. Spleen erythrocytes were lysed with ammonium chloride, and single cell suspensions were washed in HBSS. The isolation procedure for intestine lamina propria lymphocytes has been described in detail before (13, 14). For the ELISPOT assay, cells were used without further purification. For FACS analysis, lamina propria lymphocytes were purified on a 40%/100% discontinuous Percoll (Pharmacia, Uppsala, Sweden) gradient.

FACS analysis

Phenotypic analyses were performed on freshly isolated cells. FITC- or PE-conjugated Abs from PharMingen (San Diego, CA) were used at optimal concentrations. Cells at 3 to 10 × 10^5/100 μl 1% BSA/PBS were incubated with the different mAbs for 30 min at 4°C, and thereafter washed thoroughly in 1% BSA/PBS before analysis on an EPICS Profile II (Coulter, Hialeah, FL). Analyses were performed on live lymphocytes excluding dead cells and nonlymphoid cells as judged by forward and side scatter.

Serum and intestinal secretion Igs

Easy-wash 96-well microtiter plates (Corning Inc., Corning, NY) were coated with 5 μg/ml goat anti-mouse isotype- or IgG subclass-specific Abs (Southern Biotechnology, Birmingham, AL) in carbonate buffer (pH 9.5) and incubated overnight at 4°C. After washing in PBS/0.05% Tween-20 and blocking with 1% BSA in PBS for 1.5 h at 37°C, serial dilutions of sera or intestinal washings, or a mouse reference serum (ICN Biomedicals, Inc., Costa Mesa, CA) with known concentrations of the different isotypes and subclasses, were added and the plates were incubated at 37°C for 2 h. After washing in PBS/0.05% Tween-20, alkaline phosphatase-conjugated goat anti-mouse isotype- or IgG subclass-specific Abs (Southern Biotechnology) were added and plates were incubated at 37°C for 1 to 2 h. Plates were then developed using *p*-nitrophenyl phosphate (NPP) tablets (Sigma Chemical Co.), 1 mg/ml in diethanolamine buffer, pH 9.8, and the absorbance at 405 nm was determined using a Dynatech MRX ELISA reader (Chantilly, VA). Concentrations of Igs in the sera or intestinal secretions were calculated from the plotted standard curve of serial dilutions of the mouse reference serum.

ELISPOT assay

The ELISPOT assay was performed essentially as described (14). Polystyrene petri dishes (Nunc, Roskilde, Denmark) were coated with 5 μg/ml anti-mouse isotype-specific Abs (Southern Biotechnology) in PBS at 4°C overnight. After careful washing in PBS, the petri dishes were blocked with PBS/10% heat-inactivated FCS for 30 min at 37°C. Thereafter, serial dilutions of spleen or lamina propria lymphocytes (starting at 4 × 10^5 cells) in 400 μl of complete medium containing RPMI (M.A. Whittaker Bioproducts, Walkersville, MD) supplemented with 10% heat-inactivated FCS (M.A. Whittaker Bioproducts), 15 mM HEPES (Life Technologies, Grand Island, NY), 5% NCTC 109 medium (M.A. Whittaker Bioproducts), 5 × 10^{-5} M 2-ME (Sigma Chemical Co.), 2 mM glutamine (Life Technologies), and 100 U/ml penicillin/100 μg/ml streptomycin (Life Technologies) were added to each petri dish and incubated for 3.5 h at 37°C in 6% CO_2. After washing in PBS/0.05% Tween-20, the petri dishes were incubated with biotinylated isotype-specific goat anti-mouse Abs (Southern Biotechnology) (1:500) at 4°C overnight, followed by HRP-avidin at 1:500 dilution for 2 h at room temperature. Single Ab-secreting, spot-forming cells (SFC), were visualized by adding *p*-phenylenediamine (0.5 mg/ml)-0.01% H_2O_2 substrate in 1% agar in PBS to each petri dish. SFC were analyzed and counted under low magnification using a dissecting microscope (Nikon, Melville, NY).

Intestinal tissue cultures

After careful removal of Peyer's patches and fecal contents, small and large intestines were opened longitudinally and cut into 5-mm pieces. The tissue pieces were extensively washed ten times by briefly incubating in HBSS and pouring over a nylon net to retrieve the tissue pieces from the wash solution. The intestinal pieces were incubated in 24-well plates, one piece per well in 1 ml of complete medium with the addition of 0.5% Fungizone. After 24 h of culture, the supernatants were sterile filtered and stored at −70°C until analysis.

Cytokine assays

The IL-2, IL-4, IL-5, IL-6, IL-10, IFN-γ, and TNF-α content in intestinal tissue cultures were measured with specific ELISAs using the following protocol. Easy-wash ELISA plates (Corning) were coated with cytokine-specific capture Abs (2–5 μg/ml) in carbonate buffer (pH 9.5) at 4°C overnight. After washing in PBS/0.05% Tween-20 and blocking with 1% BSA/PBS for 90 min at 37°C, the plates were incubated with serial dilutions of culture supernatants or recombinant cytokines at 37°C for 2 h or at 4°C overnight. After washing, biotinylated mAbs (IL-2, IL-5, IL-6, IL-10, and TNF-α) or polyclonal rabbit anti-mouse antiserum (IL-4, IFN-γ) were added and the plates were incubated at 37°C for 2 h followed by an additional 2-h incubation with alkaline phosphatase-conjugated streptavidin (Southern Biotechnology) or goat anti-rabbit Abs (Southern Biotechnology). Finally, *p*-nitrophenyl phosphate (*p*-NPP) tablets (Sigma Chemical Co.) 1 mg/ml in diethanolamine buffer, pH 9.8, was added to each well. Color was allowed to develop at room temperature, and the plates were read in a Dynatech MRX ELISA reader at OD 405 nm. The concentrations of cytokines in the supernatants were calculated from the plotted standard curve of serial dilutions of recombinant cytokines. IL-2, IL-6, IL-10, and TNF-α ELISAs were performed using Ab pairs from PharMingen. For IFN-γ, the capture Ab (R4-6A2) was purchased from PharMingen, and a polyclonal rabbit anti-mouse IFN-γ antiserum was used for detection, as described (12). For the IL-4 ELISA, a monoclonal anti-mouse IL-4 Ab (11B11) was used as capture Ab and a polyclonal rabbit-anti-mouse IL-4

Table I. *Frequency of different T cell populations in lymphoid tissues of Gαi2-deficient and wild-type mice as determined by flow cytometry*[a]

		$CD4^+$ (%)	$CD8^+$ (%)
Large intestine			
Inbred 129/SV	Gαi2 −/− colitis	**54.9 ± 0.8**[b,c]	19.6 ± 5.1
	wild type	30.8 ± 5.2	8.7 ± 2.1
Crossbred 129/Sv × C57BL/6	Gαi2 −/− no colitis	**32.1 ± 5.0**	8.2 ± 0.6
	wild type	16.2 ± 2.4	7.5 ± 1.8
Small intestine			
Inbred 129/SV	Gαi2 −/− colitis	30.4 ± 6.1	40.7 ± 13.0
	wild type	36.5 ± 3.8	18.8 ± 6.6
Crossbred 129/SV × C57BL/6	Gαi2 −/− no colitis	29.3 ± 3.5	18.5 ± 3.3
	wild type	25.8 ± 2.7	15.8 ± 3.7
MLN			
Inbred 129/Sv	Gαi2 −/− colitis	**61.7 ± 2.7**	17.3 ± 3.0
	wild type	51.7 ± 3.2	17.3 ± 1.9
Crossbred 129/Sv × C57BL/6	Gαi2 −/− no colitis	**49.8 ± 2.3**	20.3 ± 1.4
	wild type	38.2 ± 3.3	20.6 ± 1.8
Spleen			
Inbred 129/SV	Gαi2 −/− colitis	38.7 ± 4.4	11.4 ± 2.2
	wild type	32.5 ± 1.8	12.6 ± 1.6
Crossbred 129/SV × C57BL/6	Gαi2 −/− no colitis	23.7 ± 4.4	7.9 ± 2.1
	wild type	19.6 ± 2.3	12.9 ± 2.3

[a] Lymphocytes isolated from naive 129/Sv inbred (with colitis) or 129/Sv × C57BL/6 crossbred (no colitis) Gαi2-deficient mice, and their respective sex- and age-matched wild-type mice, were stained with FITC-conjugated anti-mouse CD8 and PE-conjugated anti-mouse CD4 mAbs.
[b] The results are shown as means ± SEM from three to seven independent experiments.
[c] Data shown in bold type were found by statistical analysis to be significantly different from the corresponding values from wild-type mice.

antisera was used for detection. The rabbit IL-4 antiserum was prepared by immunizing rabbits three times (10 days apart) with 10 μg of recombinant mouse IL-4 (yeast rIL-4) (kind gift of Dr. William E. Paul, National Institutes of Health) conjugated to keyhole limpet hemocyanin in RIBI adjuvant (RIBI Immunochem Research, Inc., Hamilton, MT). The rabbits were immunized two more times with 10 μg of unconjugated rIL-4. Anti-IL-4 antiserum was shown to block rIL-4 activity in a CT.4S bioassay, and detected rIL-4 at 10 pg/ml in a sandwich ELISA at a dilution of 1:500. The specificity of this antiserum was demonstrated by the absence of reactivity against rIL-1, rIL-2, rIL-3, rIL-5, rIL-6, rIFN-γ, rGM-CSF, and rM-CSF in ELISA assays. For IL-1β, levels were determined using a kit (Endogen Inc., Cambridge, MA), according to the manufacturer's instructions.

Intestinal RNA extraction

Total RNA was isolated from intestinal tissue following snap-freezing in liquid nitrogen. Frozen tissue was homogenized with a mortar and pestle into RNAzol B (Biotecx Laboratories, Inc., Houston, TX) and vortexed vigorously to solubilize all visual clumps of tissue. The RNA were subsequently extracted with chloroform, precipitated with isopropanol, and washed with ethanol according to the manufacturer's protocol. Integrity and amount of extracted RNA was assessed by adding an equal volume of loading buffer (100% formamide, 0.25% xylene cyanol, and 0.25% bromophenol blue) to the RNA, heating at 65°C for 30 s and running on a 1.5% agarose gel containing ethidium bromide.

Analysis of IL-12 p40 mRNA by RT-PCR

Serial twofold dilutions of RNA (250 ng to 1 μg) were reverse-transcribed at 42°C for 15 min in 20 μl buffer containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 5 mM $MgCl_2$, 1 mM dNTPs, 0.75 μM oligo$(dT)_{16}$, 20 U RNase inhibitor and 50 U reverse transcriptase (all from Perkin-Elmer Cetus, Norwalk, CT). The reaction was stopped by heat inactivation for 5 min at 99°C. Subsequently, the cDNA mixture was amplified by PCR in 75 μl of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.26 mM dNTPs, and 250 ng each of specific 5′ and 3′ primer. The IL-12 p40 primer sequences were provided by Dr. Lars Eckman, University of California, San Diego, La Jolla, CA (5′-AGTGAACCTCACCTGTGACACGCC-3′ and 5′-GTTTC TTTGCACCAGCCATGAGC-3′) (15) and were synthesized by Genosys Biotechnologies, Inc. (The Woodlands, TX). The G3PDH primer set was purchased from Clontech Laboratories (Palo Alto, CA). After heating the reaction mix at 95°C for 1 min 30 s, AmpliTac DNA Polymerase (Perkin-Elmer Cetus) at 2.5 U/100 μl was added and the specific product were amplified using the following protocol: 35 cycles of 45 s denaturation at 95°C and 60 s annealing and extension at 60°C. PCR products were separated in a 1.5% agarose gel and visualized by ethidium bromide staining. To ensure that the PCR products were not the result of genomic DNA or PCR contamination, control reactions were performed in which reverse transcriptase or RNA, respectively, were omitted.

Immunohistochemistry

Expression of MHC class I and II Ags, CD3, CD4, and CD8 were examined by immunohistochemical staining of frozen tissue sections using a modified avidin-biotin peroxidase method, as previously described (16). Six-micrometer sections were fixed in cold acetone for 6 min, stored at −80°C, and prewarmed to room temperature for at least 2 h before use. The following primary Abs were used: hamster anti-mouse CD3 (clone 500-A2, Caltag Laboratories, South San Francisco, CA), rat anti-mouse CD4 (clone YTS 191.1, Caltag), rat anti-mouse CD8 (clone CT-CD8a, Caltag), biotinylated murine anti-$H2K^b$ (clone AF6-88.5, PharMingen), biotinylated murine anti-$I\text{-}A^b$ (clone AF6-120.1, PharMingen), and as a control for the latter two Abs biotinylated mouse IgG2a (anti-TNP) (PharMingen).

Endogenous avidin binding activity was blocked by preincubation with avidin D followed by biotin solutions (Vector Laboratories, Burlingame, CA), according to the manufacturer's instructions. In addition, nonspecific background and staining of endogenous mouse Igs was eliminated by pretreatment with blocking reagents from the HistoMouse SP kit (Zymed Laboratories Inc., South San Francisco, CA), following the manufacturer's instructions. Sections were incubated with primary Abs at 5 μg/ml diluted in PBS with 1% BSA at room temperature for 1 h. For nonbiotinylated primary Abs, sections were subsequently washed three times in PBS and incubated with biotinylated goat anti-hamster (1:500, Caltag) or goat anti-rat (1:1000, Caltag) Abs diluted in PBS with 1% BSA at room temperature for 1 h. All sections were then incubated for 1 h with avidin-biotin complex (Vector Laboratories) and developed with a 10 min incubation in a 50 mg/100 ml solution of diaminobenzidine in Tris buffer (pH 7.5) activated with a 1:1000 dilution of 30% H_2O_2 (16). Sections were counterstained with methyl green-Alcian blue and then dehydrated and mounted.

Statistical analysis

Student's *t* test for unmatched data was used for analysis of significance.

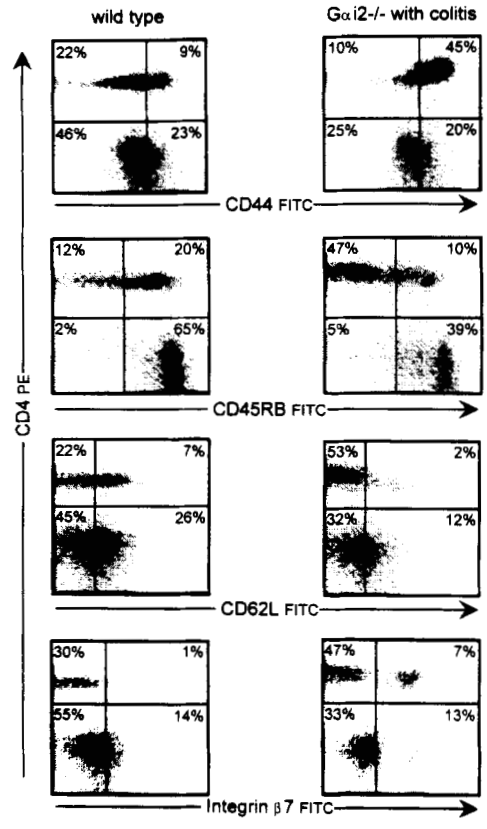

FIGURE 1. Expression of CD44 (Pgp-1), CD45RB, CD62L, and Integrin $\beta 7$ on $CD4^+$ T lymphocytes in large intestine lamina propria of Gαi2-deficient mice with colitis vs sex- and age-matched wild-type controls, as determined by flow cytometry. Data shown are from one experiment of three giving similar results.

Results

Memory $CD4^+$ T cells are increased in the large intestine lamina propria of Gαi2-deficient mice with colitis

The phenotype of lymphocytes in small and large intestine lamina propria, mesenteric lymph node (MLN), and spleen of Gαi2-deficient mice were investigated by flow cytometry and immunohistochemistry. Immunohistochemical staining with anti-CD3 demonstrated that Gαi2-deficient mice had a marked increase in T cells (56 ± 28 cells/HPF) in the large intestine as compared with wild-type mice (10 ± 5 cells/HPF). Staining with CD4 and CD8 demonstrated that the majority of infiltrating T cells in the colons of Gαi2-deficient mice were $CD4^+$ (81%). The majority of these infiltrating $CD4^+$ cells were within the lamina propria, with occasional $CD4^+$ cells seen in crypt and surface epithelium. $CD8^+$ cells represented a smaller proportion of infiltrating T cells (19%), except in the epithelium where they outnumbered $CD4^+$ cells.

By flow cytometry, the proportion of $CD4^+$ T cells in the large intestine lamina propria of Gαi2-deficient mice was confirmed to be significantly increased (Table I and Fig. 1) ($p < 0.05$). The increase in $CD4^+$ T cells in large intestine lamina propria was seen as early as 6 to 8 wk of age and persisted for as long as 1 yr in those animals surviving that long. A significant increase in $CD4^+$ T cells was also seen in the MLN, although not in the small intestine lamina propria or in the spleen (Table I). However, in the case of these tissues, the increase in $CD4^+$ T cells was age-related, being seen in older but not younger mice (data not shown). Interestingly, the increase in $CD4^+$ T cells in the large intestine lamina propria and in the MLN was seen not only in Gαi2-deficient mice with colitis, but also in Gαi2-deficient mice that did not develop colitis (Table I).

Also of interest, an increase in the frequency of $CD8^+$ T cells was seen both in the large and small intestine lamina propria of Gαi2-deficient mice with colitis. However, no changes were seen in the frequency of $CD8^+$ T cells in MLN or spleen, nor in any tissue of Gαi2-deficient mice without colitis (Table I). In preliminary studies, an increased frequency of $CD8^+$ T cells in the large intestine intraepithelial lymphocytes (IEL) of Gαi2-deficient mice was seen, both in the presence and absence of colitis (data not shown). Finally, no differences have been observed between Gαi2-deficient mice and wild-type mice in the frequency of $\gamma\delta$ TCR^+ cells in spleen, MLN, small intestine, or large intestine lamina propria (data not shown).

$CD4^+$ T cells from Gαi2-deficient mice with colitis display an increase in memory phenotype, i.e., $CD44^{high}CD45RB^{low}$, as compared with wild-type animals. The most dramatic shift in phenotype was seen in $CD4^+$ T cells from large intestine lamina propria, where both the increased frequency of $CD44^+$ T cells and the decreased frequency of $CD45RB^+$ T cells was found to be statistically significant. Also, $CD4^+$ T cells from the MLN showed increased expression of CD44 and decreased expression of CD45RB, although not statistically significant (Table II). In contrast, the increased frequency of $CD4^+$ T cells in the spleen was not associated with an increase in memory phenotype. In fact, in some instances splenic $CD4^+$ T cells displayed an increase in the $CD45RB^{high}$ phenotype. Incidentally, no alteration was seen in expression of the activation marker CD69 on $CD4^+$ T cells in any lymphoid tissue of Gαi2-deficient mice, including large intestine lamina propria (data not shown).

Increased expression of mucosal homing receptors on $CD4^+$ T cells of Gαi2-deficient mice

We next looked at expression of different adhesion molecules or homing receptors on lymphocytes from Gαi2-deficient mice. The frequency of $CD4^+$ T cells expressing CD62L (L-selectin), a marker for naive cells, but also a homing receptor for systemic lymphoid tissues (17), was substantially decreased in all tissues examined from Gαi2-deficient mice with disease, as compared with wild-type mice (Table II and Fig. 1). While only a small percentage of $CD4^+$ T cells in wild-type small intestine lamina propria expressed CD62L, the percentage was even lower in Gαi2-deficient mice. In Gαi2-deficient mice without colitis, a trend toward decreased CD62L expression was also seen in the large intestine, mesenteric lymph nodes, and spleen, although it was less dramatic (data not shown). Moreover, an increase in the frequency of $CD4^+$ T cells expressing the mucosal homing receptor integrin $\beta 7$ was seen in large intestine lamina propria and MLN ($p < 0.05$) (Table II and Fig. 1) In the small intestine lamina propria at best, a slight increase in the expresssion of $\beta 7$ was seen, and in the case of spleen $CD4^+$ T cells, either no change or a small decrease was seen. Integrin $\beta 7$ is found in association with either of two α subunits: $\alpha 4\beta 7$ (LPAM-1) or αIEL$\beta 7$ (18, 19). When the expression of integrin $\alpha 4$ was examined, no consistent differences were seen in expression of $\alpha 4$ on $CD4^+$ T cells in the large and small intestine or MLN. However, $\alpha 4$ was decreased on spleen $CD4^+$ T cells (Table II). In contrast, αIEL was significantly increased on $CD4^+$ T cells in the large intestinal lamina propria (from 6% in wild-type mice to 16% in Gαi2-deficient mice) and MLN (from 5% in wild-type mice to 13% in Gαi2-deficient mice), but only slightly elevated on spleen $CD4^+$ T cells (from 6% in wild-type mice to 8% in Gαi2-deficient mice). These changes in expression of adhesion molecules suggest changes in the homing pattern of lymphocytes,

Table II. *Expression of activation markers and homing receptors on CD4⁺ T cells from Gαi2-deficient mice*[a]

	CD44⁺[b] (%)	CD45RB⁺ (%)	CD62L⁺ (%)	Int.β7⁺ (%)	Int.α4⁺ (%)
Large intestine					
Gαi2 −/−	**62.1 ± 9.5**[c,d]	**17.4 ± 3.9**	7.8 ± 3.3	**16.1 ± 2.3**	7.9 ± 2.8
Wild type	26.1 ± 4.3	46.5 ± 8.4	22.6 ± 10.3	6.5 ± 1.3	6.6 ± 2.8
Small intestine					
Gαi2 −/−	41.6 ± 27.9	35.1 ± 15.0	2.1 ± 0.1	31.6 ± 9.1	6.0 ± 2.9
Wild type	47.1 ± 1.1	40.0 ± 13.9	6.6 ± 2.1	27.4 ± 4.0	7.7 ± 4.6
MLN					
Gαi2 −/−	15.9 ± 2.6	59.0 ± 3.5	**40.7 ± 6.6**	**11.9 ± 1.8**	7.8 ± 1.8
Wild type	11.8 ± 1.7	67.9 ± 2.2	63.0 ± 7.5	5.1 ± 0.5	6.3 ± 1.5
Spleen					
Gαi2 −/−	18.6 ± 4.9	62.8 ± 4.2	**29.3 ± 7.8**	5.9 ± 1.4	8.0 ± 3.3
Wild type	18.9 ± 5.1	57.1 ± 3.8	62.0 ± 9.9	7.2 ± 0.7	14.6 ± 3.0

[a] Lymphocytes isolated from Gαi2-deficient mice with colitis and sex- and age-matched wild-type mice were stained with specific FITC- or PE-conjugated mAbs as indicated.
[b] The frequencies of CD4⁺ T cells staining positive for different activation markers or homing receptors are expressed as the fraction of CD4⁺ T cells in each tissue.
[c] The results are shown as means ± SEM from three to seven independent experiments, except for expression of CD44, CD45RB, and CD62L on small intestine lamina propria lymphocytes, which is based on two separate experiments.
[d] Data shown in bold type were found by statistical analysis to be significantly different from the corresponding values from wild-type mice.

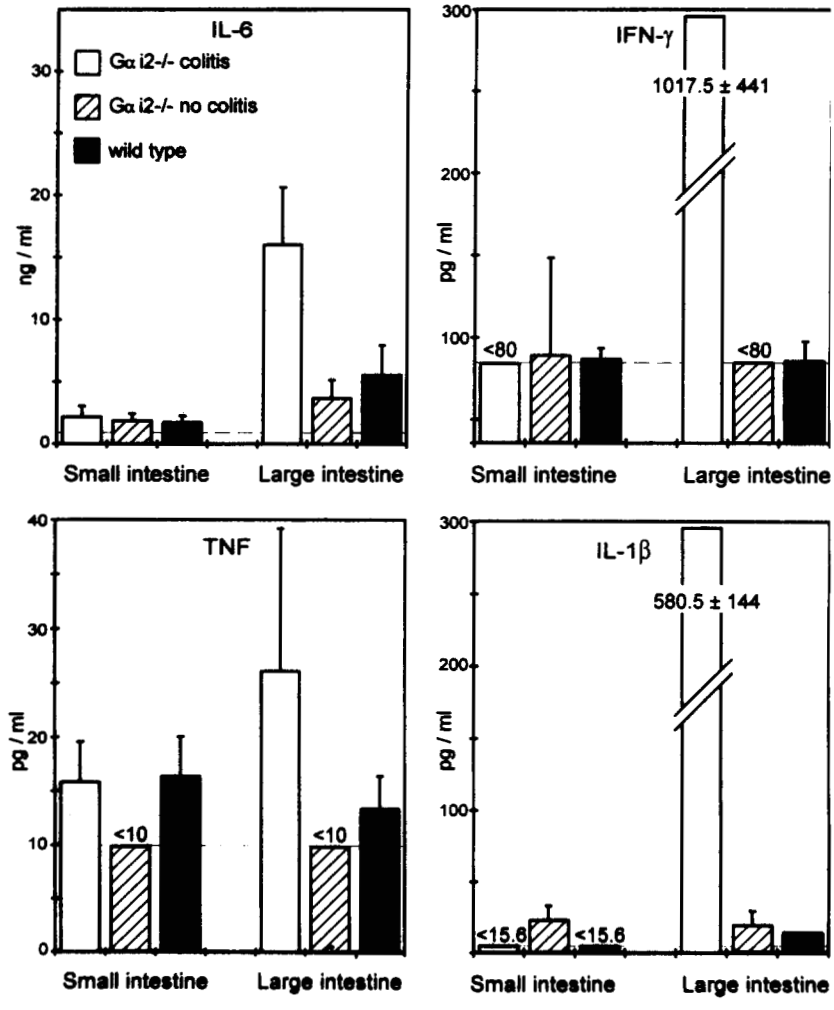

FIGURE 2. Spontaneous cytokine production in large and small intestinal tissue of Gαi2-deficient mice vs sex- and age-matched wild-type controls. Tissue culture supernatants were analyzed for cytokine content following 24 h of incubation using cytokine-specific ELISAs. The results are given as means ± SEM from ten Gαi2-deficient mice with colitis (Gαi2 −/− colitis), three Gαi2-deficient mice without colitis (Gαi2 −/− no colitis) or 17 wild-type mice analyzed individually. Each experiment included one mouse with colitis and one or more wild-type controls, and in some experiments, Gαi2-deficient mice without disease.

leading to increased homing to intestinal mucosa rather than systemic lymphoid tissues.

Increased production of proinflammatory cytokines in the colon of Gαi2-deficient mice with colitis

We measured the spontaneous production of cytokines, including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, TNF, and IFN-γ by intestinal tissues. The production of IL-1β, IL-6, IFN-γ, and TNF were significantly increased in the colons of Gαi2-deficient mice with disease. The highest increase was seen for IFN-γ and IL-1β, which were increased on average 13-fold ($p < 0.01$) and 30-fold ($p < 0.01$), respectively, as compared with wild-type mice (Fig. 2). IL-6 production was increased 5-fold ($p < 0.05$) and TNF production on average 2-fold ($p < 0.05$) above the levels found in wild-type

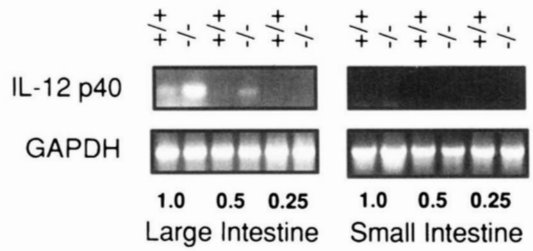

FIGURE 3. Expression of IL-12 p40 mRNA in large and small intestinal tissue of Gαi2-deficient mice with colitis and sex- and age-matched wild-type controls. Total cellular RNA (0.25–1.0 μg) was reverse transcribed and amplified by PCR using IL-12- or G3PDH-specific primers. Levels of IL-12 p40 mRNA were assessed in the large and small intestine of Gαi2-deficient and wild-type mice. Shown is the result of one experiment out of six giving similar results.

mice (Fig. 2). Of note, Gαi2-deficient mice without colitis did not show spontaneous cytokine production. While there were substantial differences in cytokine production between Gαi2-deficient mice with and without colitis, these differences were not statistically significant due to the lower number of Gαi2 −/− mice without colitis examined. Furthermore, increased cytokine production was not detected in small intestinal tissue of Gαi2-deficient mice. Neither IL-2, IL-4, IL-5, nor IL-10 were detectable in large or small intestinal tissue cultures from Gαi2-deficient or wild-type mice. Thus, Gαi2-deficient mice with colitis show a dramatic increase in the spontaneous production of Th1-type proinflammatory cytokines, but not Th2-type cytokines.

To determine whether increased IFN-γ production in the colons of Gαi2-deficient mice was a consequence of increased secretion by lamina propria lymphocytes, purified large intestine lamina propria lymphocytes were obtained and spontaneous secretion of IFN-γ was assessed. Again, a large increase in spontaneous secretion of IFN-γ was seen in the large intestine of Gαi2-deficient mice with colitis (125 ± 32 pg/ml), as compared with lymphocytes from wild-type large intestine or from small intestine of either Gαi2-deficient or wild-type mice (<40 pg/ml). This indicates that elevated IFN-γ levels in diseased colon tissue of Gαi2-deficient mice result from increased production by lamina propria lymphocytes.

IL-12, which is produced by Ag presenting cells, particularly macrophages and dendritic cells, directs T cell development towards a Th1 phenotype (20–22). We therefore examined the expression of IL-12 p40 mRNA in intestinal tissues of Gαi2-deficient and wild-type mice by semiquantitative RT-PCR. Six Gαi2-deficient mice with colitis and seven wild-type mice were analyzed for IL-12 p40 mRNA expression. We found a two- to threefold increase in IL-12 p40 mRNA in colonic tissue of Gαi2-deficient mice with colitis, as compared with wild-type mice (Fig. 3). While small, the increase in IL-12 p40 mRNA in inflamed colons was consistently seen in six separate PCR reactions utilizing RNA from the colons of 6 Gαi2-deficient and 7 wild-type mice. No increase in IL-12 p40 mRNA was seen in small intestinal tissue of Gαi2-deficient in comparison with wild-type mice. Thus Gαi2-deficient mice with colitis have increased levels of mRNA for the Th1-inducing cytokine IL-12, and increased production of IFN-γ, IL-1β, IL-6, and TNF in the large but not small intestine in comparison to wild-type mice.

Increased expression of MHC class I and class II Ags on colonic epithelium of Gαi2-deficient mice

Given the elevated IFN-γ levels present in colons of Gαi2-deficient mice, we evaluated expression of MHC class I and class II

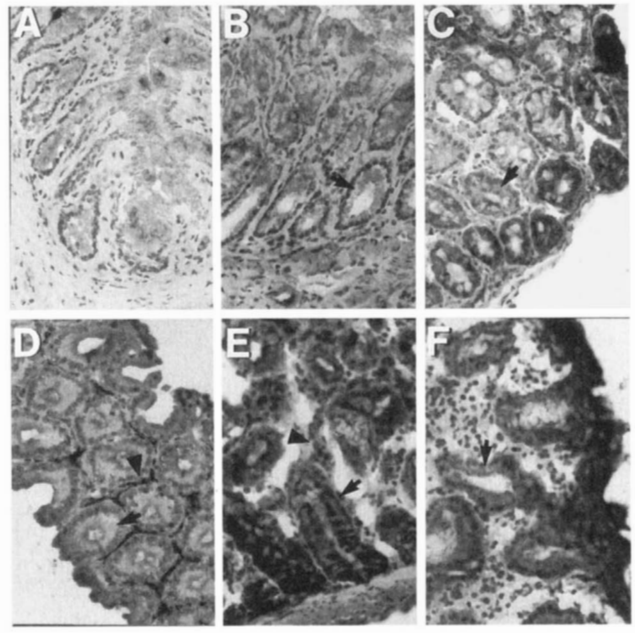

FIGURE 4. Expression of MHC class I and class II antigens on large intestinal epithelium (magnification, ×40). Frozen tissue sections from large intestine were stained with Abs against H-2K^b and I-A^b then analyzed by immunohistochemistry. *A*, control: staining with biotinylated mouse IgG2a. *B*, wild type, MHC class I: staining with biotinylated anti-H-2K^b (IgG2a). *C*, Gαi2-deficient, MHC class I: staining with biotinylated anti-H-2K^b (IgG2a). *D*, wild type, MHC class II: staining with biotinylated anti-I-A^b (IgG2a). *E* and *F*, Gαi2-deficient, MHC class II: staining with biotinylated anti-I-A^b (IgG2a). Arrows in *B*, *C*, *D*, *E*, and *F* indicate large intestine epithelial cells. Arrowheads in *D* and *E* indicate MHC class II staining in the lamina propria.

Ags in the gastrointestinal tract of these mice. Frozen sections of colons from Gαi2-deficient mice with colitis and wild-type mice were stained with Abs specific for H-2K^b and I-A^b (Fig. 4). In the case of MHC class I, wild-type mice exhibited some expression of H-2K in the lamina propria and surface epithelium, but little expression in crypt epithelium. In contrast, Gαi2-deficient mice exhibited a significant increase in expression of H-2K, particularly on crypt epithelium. In the case of MHC class II, wild-type mice exhibited substantial expression of I-A in the lamina propria, but low levels on intestinal epithelium. Again, Gαi2-deficient mice exhibited a significant increase in expression of I-A on colonic epithelium. Small intestine also showed an increase in expression of MHC class I and II in Gαi2-deficient mice, although to a lesser extent (data not shown).

Increased production of Igs in the large intestine of Gαi2-deficient mice

It was previously demonstrated that Gαi2-deficient mice have increased levels of IgG and IgM in large intestinal secretions (10). Because of the intense inflammation and increased cytokine production, notably IFN-γ, seen in the large intestine of Gαi2-deficient mice with colitis, we further investigated the type of Igs present in intestinal secretions and their source. As previously reported, a marked increase in IgG production was seen in the large intestine, being on average 60-fold higher than in wild-type mice ($p < 0.001$) (Fig. 5). Notably, Gαi2-deficient mice without colitis did not manifest increased levels of IgG in large intestinal secretions. Furthermore, an average 20-fold increase in IgM levels was evident in Gαi2-deficient mice with colitis ($p < 0.001$), but again not in mice without colitis. Despite the dramatic increase in IgG

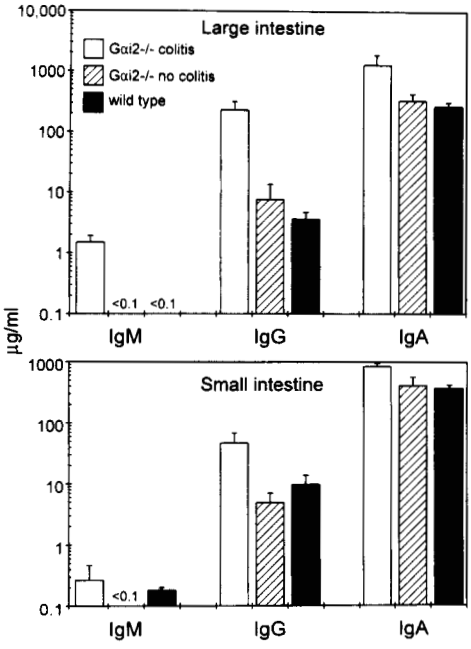

FIGURE 5. Immunoglobulin levels in intestinal secretions of Gαi2-deficient mice. Isotype-specific Ig levels were measured in intestinal lavage washings using isotype-specific ELISAs. The results are shown as means ± SEM of 8 Gαi2-deficient mice with colitis (Gαi2 −/− colitis), 3 Gαi2-deficient mice without colitis (Gαi2 −/− no colitis), or 19 wild-type mice analyzed individually.

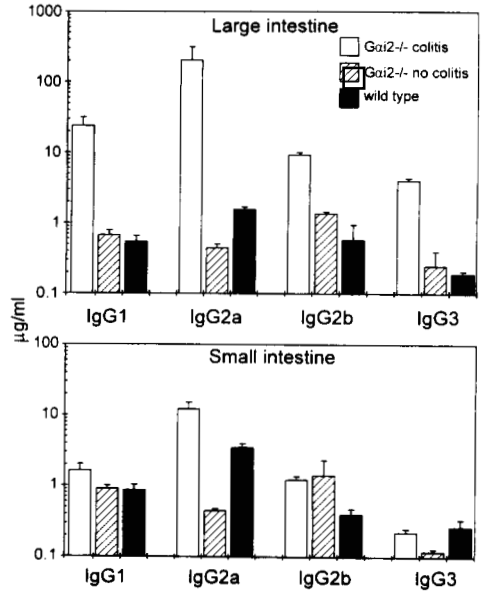

FIGURE 6. IgG subclasses in intestinal secretions of Gαi2-deficient mice. IgG subclass-specific Ig levels were measured in intestinal lavage washings using IgG subclass-specific ELISAs. The results are shown as means ± SEM of 7 Gαi2-deficient mice with colitis (Gαi2 −/− colitis), 3 Gαi2-deficient mice without colitis (Gαi2 −/− no colitis), or 15 wild-type mice analyzed individually.

levels, IgA was still the predominant isotype in intestinal secretions, with IgA levels in the large intestine of Gαi2-deficient mice with colitis being fourfold higher, as compared with wild-type mice ($p < 0.001$). Ig levels in the small intestine were less elevated in Gαi2-deficient mice in comparison to control mice, IgG levels being elevated five times in Gαi2-deficient mice with colitis, as compared with wild-type mice ($p < 0.05$).

We next investigated the levels of IgG subclasses in intestinal secretions. The highest increase was seen in IgG2a in the large intestine, being elevated up to 400-fold (on average 135-fold), as compared with wild-type mice or Gαi2-deficient mice without colitis ($p < 0.01$) (Fig. 6). IgG1, IgG2b, and IgG3 levels were also elevated from 10- to 40-fold above normal in Gαi2-deficient mice with colitis. In contrast, IgG subclass levels in the small intestine of Gαi2-deficient mice with colitis were only modestly elevated, being at most three times above normal. Thus, Gαi2-deficient mice with colitis show a large increase in IgG levels, especially IgG2a, in large but not small intestinal secretions, which correlates well with the location of inflammation.

To determine whether the increased Ig levels in intestinal secretions were due to increased local production of Igs in the mucosa, we measured the number of Ig-producing cells in the large and small intestine lamina propria using the ELISPOT technique. The number of IgG-producing cells in the large intestine lamina propria were significantly increased, on average four times in Gαi2-deficient mice with colitis ($p < 0.01$) (Table III). There was also a substantial increase in IgA-producing cells in the large intestine, but this increase was not statistically significant. This shows that the increased levels of IgG and IgA in intestinal secretions is due to elevated local production of these isotypes. Of note, an increase in IgA- and IgG-producing cells in the small intestine was also seen, although the increase was less pronounced and not found to be statistically significant. In contrast to the other isotypes, IgM-producing cells were elevated more in the small intestine than in the large intestine lamina propria.

Discussion

In this article we describe a number of dramatic changes in the phenotype and function of intestinal lymphocytes in Gαi2-deficient mice, developing acute and chronic inflammation of the colon closely resembling ulcerative colitis. Thus, the frequency of $CD4^+$ T cells expressing a memory phenotype, i.e., $CD44^{high}/CD45RB^{low}$, in the large intestine lamina propria of Gαi2-deficient mice was significantly increased. These $CD4^+$ T cells showed increased expression of the mucosal homing receptors integrins β7 and αIEL, with decreased expression of L-selectin (CD62L), a homing receptor for systemic lymphoid tissues. The Gαi2-deficient mice showed a significant increase in production of Th1 cytokines, particularly IFN-γ, in the large intestine, which was associated with an increased expression of IL-12 mRNA in the colon. In addition, we found increased expression of MHC class I and class II on large intestinal epithelial cells. Finally, colitis was associated with a significantly increased local production of IgG, especially IgG2a, in the large intestine lamina propria.

The increased frequency of memory $CD4^+$ T cells locally in the intestinal mucosa strongly suggests that these cells play a role in the pathogenesis of colitis in Gαi2-deficient mice. Studies in other animal models of IBD confirm that $CD4^+$ T cells are involved in disease pathogenesis. Thus, colitis in IL-2-deficient mice was shown to be dependent on T cells but not B cells (23), and in both the CD3ε transgenic mouse (7) and the $CD45RB^{high}$-SCID mouse (8) models of IBD, colitis could be adoptively transferred to immunodeficient recipients by transfer of splenic T cells. In addition, Rudolphi et al. have reported that repopulation of the intestine of SCID mice with $CD4^+$ T cells from an intestinal wall graft of congenic immunocompetent donor mice induced a hyperplastic and inflammatory bowel disease in recipient mice (24). Still,

Table III. *Number of Ab-secreting spot-forming cells (SFC) in the lamina propria of Gαi2-deficient mice with colitis and wild-type mice*

	IgM (SFC/10^7 MNC)	IgG (SFC/10^7 MNC)	IgA (SFC/10^7 MNC)
Large intestine			
Gαi2 −/− with colitis	3,621 ± 1,206[a]	**47,906 ± 7,791**[b]	589,062 ± 271,402
wild type	3,309 ± 2,316	12,750 ± 3,222	114,687 ± 27,254
Small intestine			
Gαi2 −/− with colitis	25,828 ± 13,019	49,812 ± 23,140	507,500 ± 105,180
wild type	9,934 ± 5,771	20,219 ± 7,372	284,375 ± 65,900

[a] Results are given as mean ± SEM of four Gαi2-deficient mice with colitis, analyzed individually, and eight wild-type control mice, analyzed in pairs. Two Gαi2-deficient mice and four wild-type mice were analyzed in two independent experiments.

[b] Data shown in bold type were found by statistical analysis to be significantly different from the corresponding values from wild-type mice.

Mombaerts et al. demonstrated that TCR α or β mutant mice, as well as MHC class II-deficient mice that lack class II-restricted $CD4^+$ T cells develop colitis, implying that a population of class II-restricted $\alpha\beta^+$ T cells is important for the prevention of colitis (4). More direct evidence for a protective role of $CD4^+$ T cells was provided by Powrie et al., who demonstrated that colitis induced in SCID mice by transfer of $CD45RB^{high}CD4^+$ T cells from normal mice was completely prevented by cotransfer of the reciprocal $CD45RB^{low}CD4^+$ T cell population (8).

Decreased expression of CD62L and increased expression of the mucosal adhesion integrins αIEL and β7 on $CD4^+$ T cells most likely influences homing of lymphocytes to mucosal tissues in these mice. Integrin β7 can associate with either α4 or αIEL (18, 19). Whereas the α4β7 receptor binds to MAdCAM-1 on endothelial cells and is involved in recruiting circulating lymphocytes into the mucosa (25), the αIELβ7 receptor binds to E-cadherin on intestinal epithelial cells and may function by retaining lymphocytes in the mucosa (26, 27). Expression of the αIEL chain is induced by TGF-β (19). It is therefore possible that in the Gαi2-deficient mice, $CD4^+$ T cells are recruited to the intestinal mucosa by their expression of α4β7, and within the mucosa the α4 chain is replaced by αIEL due to local TGF-β production. Studies of TGF-β expression and function within the mucosa of Gαi2-deficient mice might shed light on this issue.

We found a dramatic increase in the spontaneous production of Th1 cytokines in colonic tissue cultures, but no detectable production of Th2-derived cytokines. Th1-derived cytokines are proinflammatory and are likely involved in the inflammatory response in the large intestine of Gαi2-deficient mice. The key cytokine that directs the immune response toward a Th1-dominated response is IL-12 (20–22). Our data suggest that the Th1-dominated, inflammation-inducing cytokine profile in the colons of these mice is caused by an increased IL-12 production, as indicated by increased IL-12 p40 mRNA expression. IL-12 production by macrophages and dendritic cells is normally induced by bacterial products, and this induction has been shown to be enhanced by exposure to IFN-γ (28–30). We do not yet know the cause of the increased IL-12 and IFN-γ production in Gαi2-deficient mice, but it is possible that alterations in macrophage or dendritic cell function resulting from the Gαi2 deficiency induces increased IL-12 expression, which in turn leads to production of proinflammatory Th1 cytokines.

Whole intestinal tissue rather than isolated lymphocytes were used in these experiments because in initial experiments several of the cytokines could not be detected in purified lamina propria lymphocyte cultures. This most likely occurred because several of these cytokines are produced by macrophages/dendritic cells or epithelial cells rather than lymphocytes. It might be argued that the increase in cytokines seen in the large intestine of Gαi2-deficient mice is simply the result of an increased number of T lymphocytes in the inflamed lamina propria. This is not the case, since isolated large intestine lamina propria lymphocytes from Gαi2-deficient mice with colitis spontaneously secreted significantly increased amounts of IFN-γ, as compared with equal numbers of lymphocytes from control mice. Neither IL-1β, IL-6, nor TNF could be detected in large intestine lamina propria lymphocyte cultures, indicating that the elevated production of these cytokines in the large intestine of Gαi2-deficient mice occurred in nonlymphocytes, i.e., epithelial cells or macrophages. This is also consistent with the finding that IFN-γ production, but not IL-1β, IL-6, or TNF production were further increased following polyclonal T cell stimulation with anti-CD3 in vitro (not shown).

Previous studies have reported IL-1, IL-6, TNF-α, and IFN-γ, as well as the inflammatory mediators prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4), and PAF, to be increased in the mucosa of patients with ulcerative colitis (1, 31–37). In preliminary experiments, we have found increased levels of both PGE_2 and LTB_4 in supernatants from large intestinal tissue cultures from Gαi2-deficient mice with colitis.

Gαi2-deficient mice with colitis have dramatically increased expression of both MHC class I and class II Ags on their intestinal epithelium. Mayer and co-workers have reported that MHC class II^+ intestinal epithelial cells in normal subjects can function as APCs but that they selectively activate $CD8^+$ Ag-nonspecific suppressor T cells, in contrast to conventional APCs. However, enterocytes from patients with inflammatory bowel disease instead stimulate $CD4^+$ T cells that display potent T helper activity (38, 39). It is therefore possible that the increased expression of MHC molecules allows Gαi2-deficient intestinal epithelial cells to present luminal Ags to and activate $CD4^+$ T cells in the lamina propria.

The increased expression of MHC class I and especially MHC class II on the large intestinal epithelium is most probably due to increased production of IFN-γ, as such effects have previously been described (40, 41). IFN-γ can also enhance an inflammatory response by increasing mucosal permeability by affecting epithelial cell tight junctions (42) and by modulating neutrophil migration across intestinal epithelial monolayers in a CD11b/18-dependent fashion (43). It also appears to be the mediator, together with IL-2, of intestinal tissue destruction after experimental activation of lamina propria lymphocytes (3).

In parallel with what is seen in patients with IBD, we found elevated levels of IgM and especially IgG in the large intestine of Gαi2-deficient mice with colitis. Normally, IgA is the dominant isotype in intestinal mucosa with very low levels of IgG being present. In contrast to IgA, which is able to neutralize or eliminate potential pathogens in mucosal tissues without inducing inflammation, IgG is a potent activator of complement and a good opsonin. Consequently, the dramatic increase in IgG production locally in the intestinal mucosa is potentially proinflammatory.

However, in analyzing the number of Ig-producing cells within the intestinal mucosa by ELISPOT, we found that the number of IgG- and IgM-producing cells were increased both in the small and large intestine. The lack of a concomitant increase in IgG and IgM levels in small intestinal secretions and the absence of inflammation in the small intestine suggests that the increase in IgM and IgG in the colon of diseased mice may be a consequence of increased permeability secondary to, and thus not the cause of, the inflammation. In accordance with this, Ma et al. have recently shown that B cells are not required for development of IBD in IL-2 knockout mice (23). Breeding the Gαi2-deficient mice onto a B cell deficient background will provide insight regarding the contribution of B cells to development of disease.

While the nature of the genes involved in the pathogenesis of ulcerative colitis has yet to be established, evidence suggests that it is a multigenic disease with several genes being involved in disease expression (44). In accordance, we found a genetically determined susceptibility to the development of colitis in mice with a mutated Gαi2 gene. Thus, an inbred 129/Sv background was associated with a high incidence of disease in Gαi2-deficient mice, whereas a crossbred 129/Sv × C57BL/6 background was associated with a much lower incidence and severity of colitis. Nonetheless, the nature of these susceptibility genes remains to be determined. As both the C57BL/6 and the 129/Sv strains express the H-2^b MHC haplotype, the disease susceptibility gene or genes are most probably located outside of the MHC locus. The influence of multiple genes on disease expression is seen in other animal models of diseases, e.g., the NOD mouse and the EAE (experimental autoimmune encephalo-myelitis) model, in which disease expression is profoundly affected by strain background (45–47).

Numerous studies indicate that bowel microflora or their products play a role in the pathogenesis of IBD (48–50). In addition, in the IL-2 knockout mouse model and the HLA-B27 transgene rat model of IBD, the animals did not develop disease when kept in a germfree environment (5, 51). Gαi2-deficient mice were found to develop colitis and colon cancer at the same frequency irrespective of whether they were bred in a specific pathogen-free barrier facility or in a conventional animal facility. Furthermore, although the number of bacterial species in fecal samples was lower in mice maintained in the barrier facility, there were no differences in stool microflora between Gαi2-deficient and wild-type mice within the same facility (not shown). Still, it will be important to perform studies involving maintenance of Gαi2-deficient mice under germfree conditions. A new *Helicobacter* species has been identified (*Helicobacter hepaticus*) that colonizes the colons and livers of mice and can cause hepatitis (52). Stool samples from Gαi2-deficient mice with colitis were tested for the presence of *H. hepaticus* by PCR, and 5 of 10 mice were found to be positive (James Fox, Massachusetts Institute of Technology, unpublished results). Thus, it is possible that *H. Hepaticus* plays a role in development of IBD in these mice, but more extensive studies will be required to determine this.

Notwithstanding the contribution that other animal models of IBD, such as IL-2, IL-10, MHC class II, and TCR α- or β-chain knockout mice (4–6) are likely to provide, there is little evidence that IBD in humans result from a gross deficiency in a particular cytokine or T cell subset. Instead, a more subtle abnormality in mucosal immune function is likely to be involved in the pathogenesis. Given the ubiquitous nature of G proteins, there are a variety of mechanisms whereby inactivation of the Gαi2 gene might cause a defect in the regulation of immune responses and play a role in development of IBD. For example, inhibitory G proteins (Gi) proteins regulate certain events in T cell activation and thymocyte differentiation (53, 54) and have also been shown to modulate T lymphocyte homing to mucosal tissues through effects on adhesion molecule expression or function (55). Further, Gi proteins couple a variety of receptors and secondary messengers to the MAP kinase pathway (56–59), which is of significance since many cytokines, including IL-3, IL-5, GM-CSF, stem cell factor, IL-1, TNF-α, IL-2, and IL-6, have been shown to signal through the MAP kinase pathway. Recently, a carboxy-terminal splice variant of Gαi2 has been described that is localized to the Golgi apparatus and may be involved in Golgi transport and function (60). This might be important for Ag presentation or cytokine secretion by cells of the immune system. Further, receptors for chemokines such as IL-8, fMetLeuPhe and LTB_4 have been shown to be coupled to Gi-proteins (61). Finally, α-2-adrenergic and muscarinic receptors are present on normal human intestinal mucosa. These receptors are coupled to Gi2 and Gi3 which mediate inhibition of adenylate cyclase in these cells (62). Blunting of adenylate cyclase inhibition has been observed in heart homogenates (with carbachol) and adipocyte membranes (with PGE2 and nicotinic acid) derived from Gαi2-deficient mice (63). Thus, Gαi2, by transducing signals from these receptors, might regulate the function of intestinal epithelial cells. Future studies addressing these possibilities should elucidate the mechanisms of disease pathogenesis leading to colitis in Gαi2-deficient mice.

Acknowledgments

We gratefully thank Madelyn Vuong for help with breeding of the mice, Dr. Milton Finegold for performing the histopathologic analyses, Dr. Richard Brown for helpful discussion regarding immunohistochemistry, Dr. James Fox for testing for *H. hepaticus*, and Fredrik Knoop for help with illustrations.

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